Supplementary Information for

Prolonged Fasting reduces IGF-1/PKA to promote hematopoietic stem cell-based regeneration and reverse immunosuppression

Chia-Wei Cheng,[1] Gregor B. Adams,[2] Laura Perin,[3] Min Wei,[1], Xiaoying Zhou [2] Ben S.Lam,[2] Stefano Da Sacco,[3] Mario Mirisola,[4] David I. Quinn,[5] Tanya B. Dorff, [5] John J. Kopchick[6] and Valter D. Longo[1*]

Correspondence to: vlongo@usc.edu

This PDF file includes:

Supplementary Figures: S1 to S5

Supplementary Tables:S1 to S5

Supplementary materials and methods

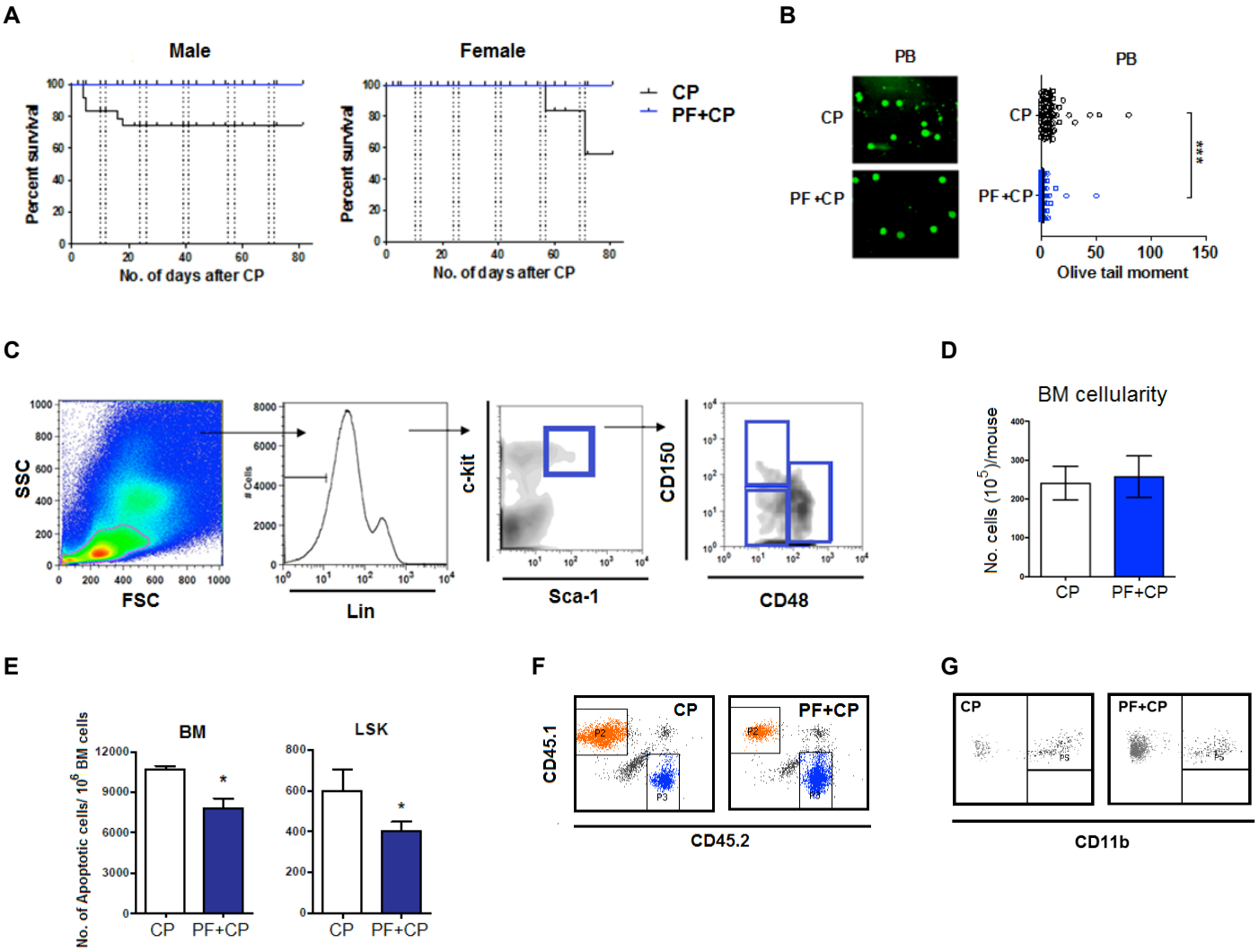

Figure S1. Protective effects of PF against chemotoxicity in hematopoietic system, related to Figure 1. (A)Survival curve of male and female mice. (B)DNA damages in mouse peripheral blood cells measured by comet assay (olive tail moment) (day 81, 6th recovery phase). (C) Gating of LT-HSC, ST-HSC and MPP. Lin- gated population with positive Sca-1 and c-kit markers are defined as LSK cells; from the LSK gated cells, LT-HSCs are CD48-CD150+, ST-HSCs are CD48-CD150- and MPPs are CD48+. (D) No. of total bone marrow cells per mouse. (E) Apoptosis analysis for bone marrow cells of CP treated mice using Annexin V and 7AAD. (F) Gating for CD45.2+ cells. The percentage of CD45.2+ cells shown in main figures is based on the number of (P3, blue) divided by the sum of number of total single positive subsets (P2 and P3).(G) Gating for myeloid cells from CD45.2+ cells (CD11b+, P5).

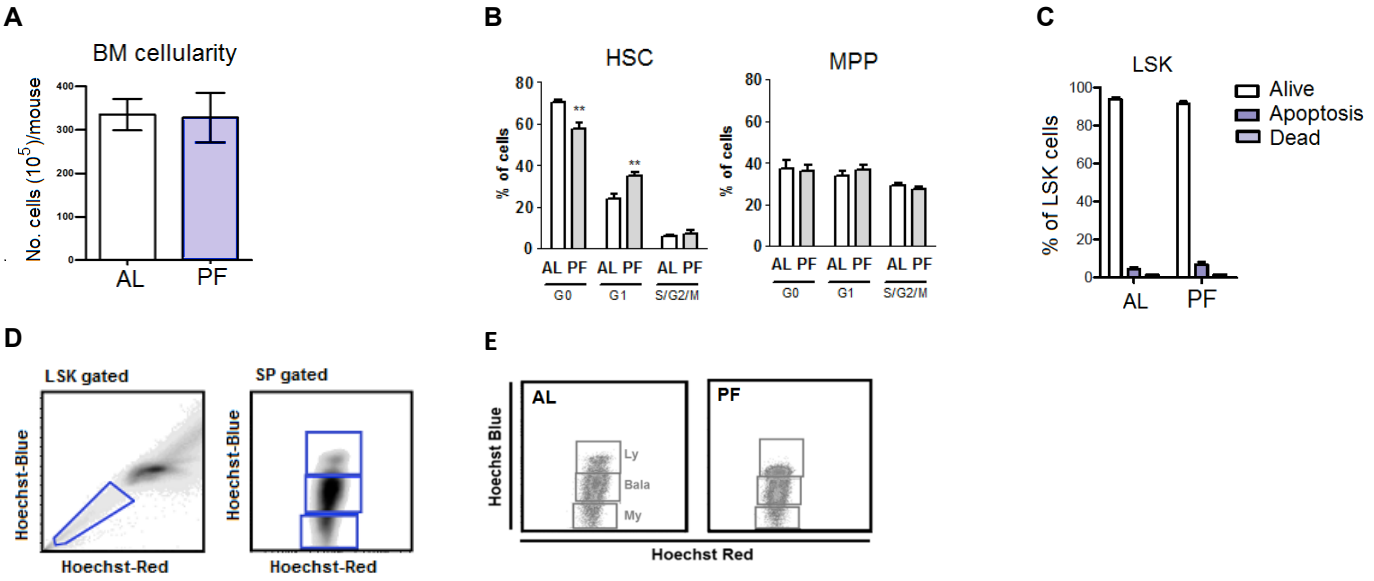

Figure S2. Effects of PF on murine bone marrow and LSK cells, related to Figure 2.
(A) Bone marrow cellularity of mice on either PF or fed *ad libitum* (AL). (B) Cell cycle analysis for HSCs and MPP using Pyronin Y/Hoechst33342 .(C) Apoptosis analysis for bone marrow cells using Annexin V and 7AAD. (D) Gating of side population: High-, Middle- and Low-SP.(E) Representative micrograph for Ly-HSC, Bala-HSC and My-HSC shown in figure 2F.

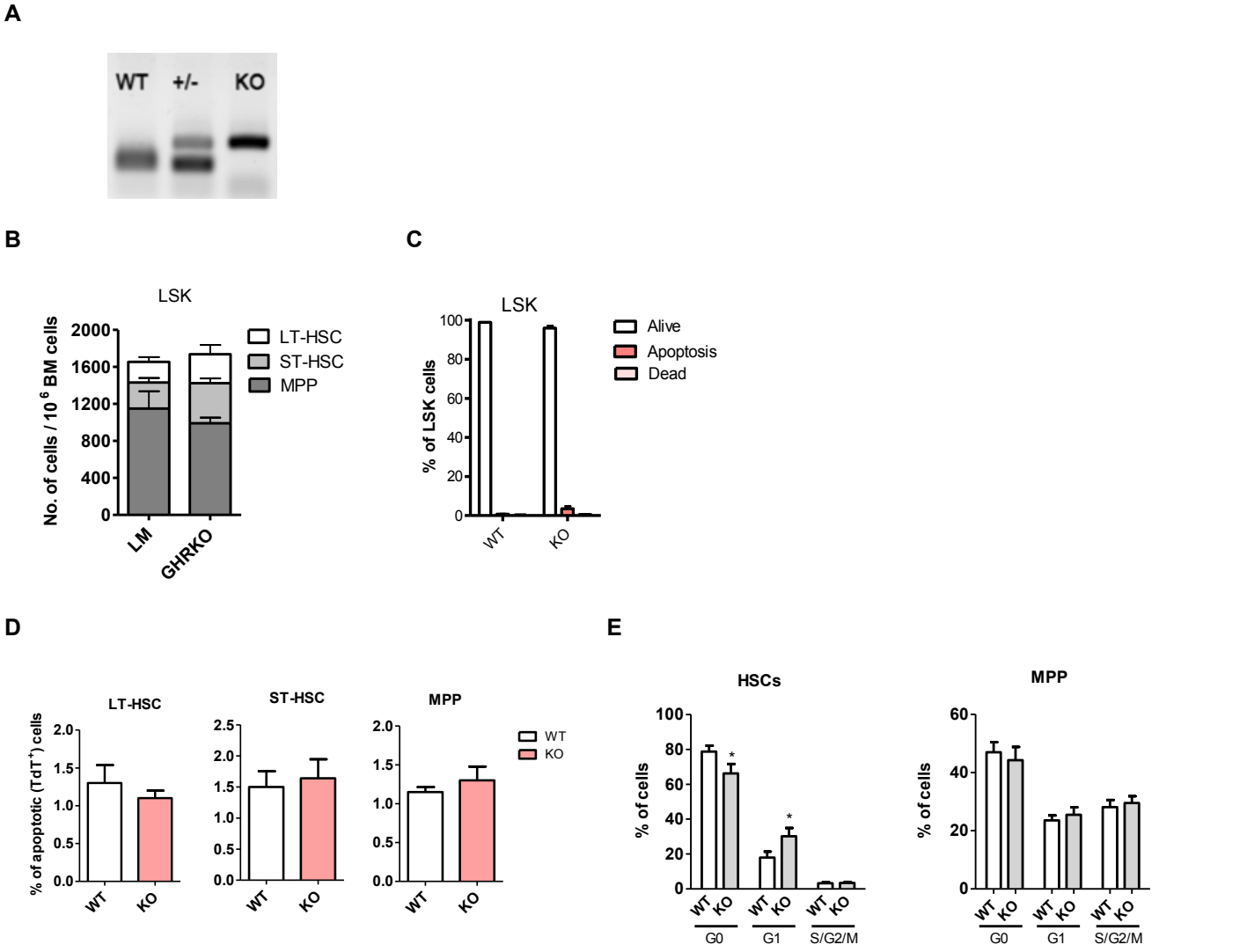

Figure S3. Effects of GH/IGF-1 deficiency on murine bone marrow and LSK cells, related to Figure 3. (A)Genotyping for GHRKO mice. Primers generate 250bp (GHR) and 350bp (disrupted GHR) bands as shown in the image of gel. (B)Number of HSCs and MPP (C) Apoptosis analysis for HSCs and MPP using Annexin V and (D) using TUNNEL assay and (E) Cell cycle analysis for HSCs and MPP using PY/Hoechst staining.

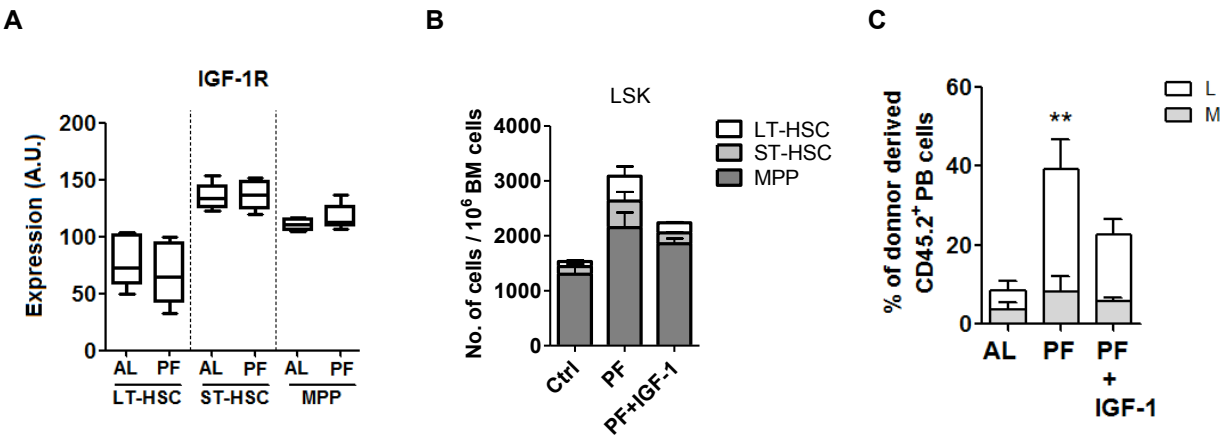

Figure S4. IGF-1-PKA signaling in mammalian cells, related to Figure 4. (A)IGF-1 receptor expression levels in HSCs and MPP. (B)No. of HSCs and MPP in bone marrow.(C)L/M ratio of donor-derived leukocytes at 16 weeks after primary transplantation.

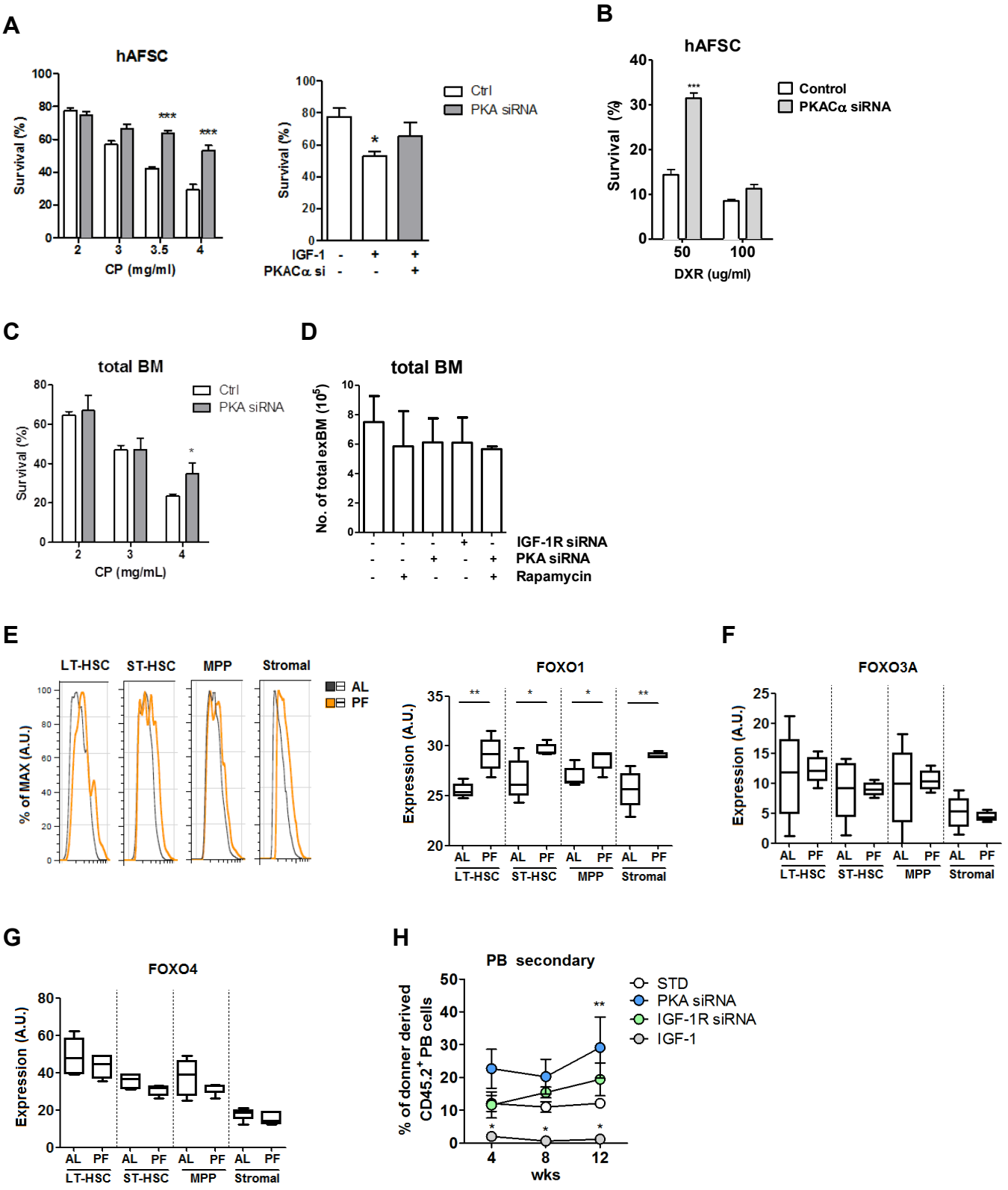

Figure S5. Reduction of IGF-1/PKA signaling mimicking fasting, related to Figure 6. (A) Survival of human amniotic fluidic stem cells (hAFSCs) treated with cyclophosphamide (CP) or (B) doxorubicin (DXR). (C) Survival of murine bone marrow under CP treatments. For (A-C), percentage of survival was normalized by the number of cells before the treatment of chemotherapy drugs. (D) Number of total BM cells with the indicated ex vivo treatments. (E-G) Expression levels of indicated proteins in hematopoietic stem/progenitors and their stromal niche cells. (H) The engraftment in PB measured after secondary transplantation.

Supplementary tables

Table S1. Treatments and the absolute lymphocyte counts (10^9/L) in patients on either 24hr or 72hr fasting, related to Figure 1.

		BL	C1D1	C1D8	C2D1	C2D8
24hr	CISPLATIN/GEMCITABINE	1.6	0.75	1.07	0.96	1.55
	CISPLATIN/GEMCITABINE/CARBOPLATIN	1.66	1.47	1.92	1.08	
	CISPLATIN/GEMCITABINE	0.52	0.98	0.31	0.53	0.44
	Carboplatin+Paclitaxel	2.26	2.46	0.81		
	CISPLATIN/GEMCITABINE	2.7	2.4	1.6	1.1	
72hr	Carboplatin+Paclitaxel	1.5	1.18		1.37	
	CISPLATIN/GEMCITABINE	2.62		*2.52*	2.05	1.95
	Carboplatin+Paclitaxel	1.9	2.3	*2*	1.8	
	CISPLATIN/TCH	3	1.2		2.5	
	Carboplatin+Paclitaxel	2.5	1.66		2.2	

Table S2. Primer set used for GHRKO mice genotyping, related to experimental procedures:mice.

Primers:
Intron4- 5'-CACACCATCCGACTGAGAAA
Intron3+ 5'-GAATGGCACATGTCCTTCCT
NeoB- 5'-CCACACGCGTCACCTTAATA

Table S3. Fold change (FC) of PRKACA mRNA level in mice after 48-hr PF, related to Figure 4.

	FC	z ratio	*P* value
Liver	-1.59	-2.07	0.015
Kidney	-1.49	-1.50	0.015
Muscle	-2.95	-2.67	<0.0001
Heart	-1.80	-2.08	<0.0001

Table S4. Expression level change of PKA related genes in IGF-I deficient serum-treated human epithelial cells, related to Figure 4.

	FC	z ratio	*P* value
Structural			
PRKAR2A	-1.13	-1.70	0.0003
Anchoring			
AKAP7	-1.05	-0.84	0.0234
PAKAP9	-1.05	-0.83	0.0397
Inhibitory			
PKIB	1.14	1.78	0.0522

Table S5. Inhibition efficiencies of the target proteins, related to Figure 6.

	Target	Reduction (%)
IGF-1R siRNA	IGF-1R	54.25±6.9
PKA siRNA	PKACα	72.49±2.3
Rapamycin	mTORC1(p-S6RP)	47.72±13

Supplementary materials and methods

Cell culture and treatments

Human amniotic fluid stem cells (hAFSCs) were grown and passaged as previously described (a-MEM with 15% ES-FBS, 1% glutamine and 1% penicillin/streptomycin, supplemented with 18% Chang B and 2% Chang C) (De Coppi et al., 2007).Human IGF-1 induction (10nM, 15min) was performed at 24hr after transfection. For stress resistance experiment, CP or Doxorubicin (DXR) treatments (2-4mg/ml) were given at 24hr after siRNA transfection; Cell survival was measured 18hr after CP or DOX treatments by the cell Titer blue viability assay (Promega).

Microarray analysis

Raw data were subjected to Z normalization and deposited in the Gene Expression Omnibus (GEO) repository (accession number GSE21980) Gene set enrichment was tested with the PAGE method as described (Guevara-Aguirre et al., 2011; Kim and Volsky, 2005; Lee et al., 2012). Tables S3 and S4 were generated based on the names and descriptions provided by IPA (Ingenuity Systems) and/or Ariadne Pathway Studio 7 (Ariadne Genomics).